

REMARKS

I. Status of the Claims

Claims 1, 4, 6, 10-27, 30-48 and 71 are pending and under consideration, claims 2, 3, 5, 7-9, 28, 29 and 49-70 having been previously canceled. With this Amendment, claims 1 and 27 are being amended. Thus, after entry of this Amendment, claims 1, 4, 6, 10-27, 30-48 and 71 remain pending and under consideration. The amendments of the claims and the various rejections raised in the Office Action are discussed in more detail, below.

Applicants understand all previous rejections, other than those discussed below, to have been withdrawn.

II. Amendments

Claims 1 and 27 are amended to specify that the seed containing the cell and the nucleic acid are placed in a container, and the pressure is reduced in the container by about 0.096 MPa from the atmospheric pressure. Basis for these amendments can be found at least in claim 70 on page 15 and in Example 1 of PCT Publication WO 2004/0046416.

No new matter is added by way of these amendments.

III. Rejections under 35 U.S.C. §103(a)

Claims 1, 4, 6, 10-27, 30-48 and 71 were rejected under 35 U.S.C. §103(a) as allegedly obvious over Rickwood (of record) in view of Dev (of record).

Claims 1, 4, 6, 8-27, 29-48 and 71 were rejected under 35 U.S.C. §103(a) as allegedly obvious over Schmukler (of record) in view of Gutierrez-Armenta (of record) and Dev.

These rejections are respectfully traversed.

A. The Present Claims

As amended, the present claims refer to a method for improving the efficiency of transfer of a nucleic acid into a plant cell having an intact cell wall and contained in a seed, comprising the steps of a) placing a seed containing the cell and the nucleic acid in a container and depressurizing the container wherein the pressure in the container is reduced by about 0.096 MPa from the atmospheric pressure; b) placing the seed containing the cell and the nucleic acid under conditions to induce electroporation; and c) transferring the nucleic acid into the plant cell using electroporation (Claim 1); and a method for improving the efficiency of introducing a nucleic acid into a cell of a plant, wherein the cell has an intact cell wall, comprising the steps of a) placing a seed containing the cell and the nucleic acid in a container and depressurizing the container

wherein the pressure in the container is reduced by about 0.096 MPa from the atmospheric pressure; b) placing the seed containing the cell and the nucleic acid under conditions to induce electroporation and introducing the nucleic acid into the cell using electroporation; and c) differentiating, growing, and/or multiplying the cell (Claim 27).

B. The Cited Art

RICKWOOD discloses a method of transfecting cells involving the generation of bubbles of gas in a liquid medium which interact with the cell to be transformed and form a hole in the cell's surface.

DEV disclose a method for producing genetically modified plants via electroporation in the absence of cell-wall degrading enzymes.

SCHMUKLER discloses an apparatus and method for electroporation and electrofusion of cells, in particular myeloma and lymphoma cells, which are types of animal cells, as well as isolated nuclei (see Col. 3 of Schmukler).

GUTIERREZ-ARMENTA discloses the use of retinoblastoma protein to control growth of plant cells and/or plant viruses. Several methods of administering nucleotides to cells are disclosed, including electroporation of plant seed cells with DNA.

C. Analysis

C1. Rejection of claims 1, 4, 6, 8-27, 29-48 and 71 as allegedly obvious over Rickwood in view of Dev

Applicants respectfully note that, in assessing the scope and content of the prior art, the references must be considered in their entirety, *i.e.*, each as a whole including portions that would lead away from the claimed invention. See *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 US 851 (1984); see also M.P.E.P. § 2141.02. In the present rejection, the Examiner appears to be failing to consider the primary reference, Rickwood, as a whole, and arriving at a finding of obviousness based only on hindsight.

The Rickwood reference is directed to the introduction of a substance into a cell not by electroporation, but by causing bubbles to interact with the surface of the cell and form holes in the cell surface. While Rickwood speculates on page 9 that the disclosed method can be applied to plant, fungal or bacterial cells having cell walls, in such instances, the method would clearly require the preparation of a protoplast from the cell to eliminate the cell wall. The Examiner appears to be relying on Rickwood solely for its general suggestion that plant protoplasts could be

transformed with the bubble method, and that the method of transfection could be performed at a pressure above or below atmospheric pressure. However, contrary to the Examiner's assertion, the presently claimed depressurization step wherein the pressure in the container is reduced by about 0.096 MPa from the atmospheric pressure is not merely a matter of optimizing the method of Rickman, as Rickman fails to teach a method of transforming a plant cell having an intact cell wall and contained in a seed. Furthermore, as argued previously, applying the method described in Rickwood to a plant cell having an intact cell wall and contained in a seed, by placing the seed containing the cell and the nucleic acid in a container -- whether or not the pressure in the container were reduced by about 0.096 MPa from the atmospheric pressure as presently claimed - - would clearly not result in transformation of the plant cell having an intact cell wall and contained in a seed. The skilled artisan would understand that Rickwood's method using bubbles to interact with a cell wall would fail to form holes in the intact cell wall of a plant cell contained within a seed.

Moreover, Rickwood teaches away from the use of electroporation for cell transformation. Specifically, those skilled in the art, when considering Rickwood's disclosure, would note and could not ignore the teaching therein that electroporation is set forth as an undesirable method for transfection. See page 1 of Rickwood, which states:

of cell. More recently there has been interest in introducing both proteins and drugs into living cells without damaging the cells. A significant problem to be overcome when developing such techniques is the general imperviousness of the cell membrane. The cell membrane is normally impervious to even small molecules, unless they are very lipophilic. Even short-term damage to the cell membrane to render it more permeable tends to result in cell-death. This is a particular problem associated with electroporation, discussed below.

On page 2, Rickwood goes on to note the inefficiency of existing treatments, in which a large proportion of cells are killed. Thus, Rickwood not only fails to contemplate the method as presently claimed, but actually teaches away from it.

In light of this teaching away from electroporation, the skilled artisan would lack any motivation to combine Rickwood with any other reference teaching electroporation, such as the secondary reference cited in this rejection, Dev. And while Dev describes transformation of desiccated seed embryos in the absence of cell wall degrading enzymes via electroporation, this reference fails to contemplate placing a seed containing the cell and the nucleic acid in a container

and depressurizing the container wherein the pressure in the container is reduced by about 0.096 MPa from the atmospheric pressure. Again, contrary to the Examiner's assertion, the presently claimed depressurization step wherein the pressure in the container is reduced by about 0.096 MPa from the atmospheric pressure is not merely a matter of optimizing the method of Rickman, as Rickman fails to teach any benefits associated with reducing the ambient environmental pressure which would be recognized by those skilled in the art as being beneficial for modifying the electroporation method as described in Dev. Finally, Rickwood would not be able to achieve the presently claimed level of depressurization, because Rickwood uses an open system (not separated from the atmosphere) rather than the presently claimed container in which the seed is placed.

Thus, in considering the disclosure of references as a whole, Applicants submit that the Rickwood reference teaches away from electroporation, and those skilled in the art at the time of the invention, without the benefit of hindsight, would not have been motivated to combine the electroporation method described in Dev with the method described in Rickwood.

Applicants respectfully request withdrawal of this rejection under 35 U.S.C. §103(a).

C2. Rejection of claims 1, 4, 6, 8-27, 29-48 and 71 as allegedly obvious over Schmukler in view of Gutierrez-Armenta and Dev

The primary reference, Schmukler, teaches a method of electroporation in which cells are trapped into pores in a film with diameters smaller than the diameters of the cells, and an electric field is applied to cause electroporation. The Examiner points to column 3, lines 44-47 as teaching exposure to depressurization across the film containing the pores. Schmukler also discloses an apparatus and method directed to electroporation and electrofusion of cells, in particular animal cells, as well as isolated nuclei (see Col . 3 of Schmukler). Schmukler fails to teach or suggest introduction of nucleic acids into plant cells, but rather, is directed to methods for fusing two types of cells, or fusing a cell with an isolated nucleus, rather than the method as presently claimed.

Applicants note that the method of cell trapping by applying a vacuum as described in Schmukler is completely different from the depressurization step of the container containing a plant seed containing a cell having an intact cell wall in which the pressure in the container is reduced by about 0.096 MPa from the atmospheric pressure of present claims 1 and 27. In the method of Schmukler, a vacuum is used to trap cell on a film having pores to retain the cell, wherein the size of the pores is smaller than the cells, and cultured single cells are retained. Like Dev, Schmukler fails to teach or suggest the presently claimed method in which a plant cell having an intact cell

wall and contained within a seed is transformed by electroporation and depressurization, and, moreover, Schmukler further fails to teach that the seed is placed within a container that is depressurized, as presently claimed.

Furthermore, it would be understood by those skilled in the art that the method of Schmukler, when applied to plant seeds containing many cells, would likely result in the cells contained within the seed being compressed, reducing spaces between cells, and thereby inhibiting nucleic acid containing buffer solution from entering said spaces between cells within the tissue. The method of Schmukler, when applied to seed tissue, would be understood to have an effect opposite to the effect of improving transfection efficiency attained by the claimed methods and described on page 32, line 26 to page 33, line 3 of the present application, PCT Publication WO 2004/007736. The method of Schmukler would likely compress the seeds and reduce the ability of the nucleic acid solution to contact the cells within the seed being placed under pressure. Furthermore, like Rickwood, Schmukler would not be able to achieve the presently claimed level of depressurization, because Schmukler's system does not require the container as presently claimed, in which the seed is placed for the depressurization.

While Gutierrez-Armenta teaches the electroporation of DNA into plant seed cells, this reference fails to teach, enable or suggest transformation of a plant cell having an intact cell wall and contained within a seed. The electroporation method taught by Gutierrez-Armenta is completely silent with respect to how a nucleic acid could penetrate an intact plant cell wall.

Accordingly, the references, alone or combined, fail to teach or enable the skilled artisan at the time of filing any reasonable expectation of success in achieving the presently claimed method.

CONCLUSION

Because the cited references, when considered either singly or in combination, fail to teach, enable or suggest the presently claimed method, and could not lead one skilled in the art to expect to achieve the presently claimed method, the standard for obviousness has not been met. Accordingly, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §103.

In view of the foregoing, claims 1, 4, 6, 10-27, 30-48 and 71 are believed to satisfy all of the criteria for patentability and are in condition for Allowance. An early indication of the same is therefore kindly requested.

No fees other than the RCE fee are believed to be due in connection with this Amendment. However, the Commissioner is authorized to charge any additional fees that may be required, or credit any overpayment, to King & Spalding LLP Deposit Account No. 50-4616.

If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 590-1932.

Respectfully submitted,
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